6. The method of claim 1, wherein prior to said introducing step, said[the] mammal has immunocompetent memory cells which are specific for [the] said immunogenic polypeptide.

8. The method of claim 7, wherein expression of the immunogenic polypeptide is inhibited *in vitro* by exposure of the cell to the regulatory drug, and wherein expression in the mammal is induced after a delay interval [, the mammal being substantially free of the] following removal of regulatory drug exposure.

9. The method of claim 7, wherein expression of the immunogenic polypeptide is inhibited *in vitro* by substantial absence of the regulatory drug and wherein expression in the mammal is induced after a delay interval by administration of the regulatory drug to the mammal [of the regulatory drug].

14. [A] An isolated cell transformed with a nucleic acid sequence encoding a polypeptide which is immunogenic to a mammal, the nucleic acid sequence being operably linked to a drug-regulatable promoter, such that expression of the immunogenic polypeptide by the cell may be controlled by altering the concentration of regulatory drug to which the cell is exposed in a mammal.

16. A composition comprising a plurality of [a cell] cells of claim 14 and a physiologically acceptable diluent.

18. A method of regulating the expression of a nucleic acid sequence encoding a heterologous polypeptide in a leukocyte, comprising [introducing into] transforming the isolated leukocyte with the nucleic acid coding sequence operably-linked to a tetracycline-operator sequence, and a sequence encoding a tetracycline-sensitive DNA-binding expression-regulating polypeptide; and altering the concentration of tetracycline [(or analogues thereof)] , or an analogue thereof, to which the leukocyte is exposed, so as to regulate expression of the coding sequence.

REMARKS

Claims 1-18 are currently pending in the application.

Rejections under 35 U.S.C. §101

Claims 14-16 stand rejected under 35 U.S.C. §101 as being directed to non-statutory subject matter. The Examiner states that the claim reads on cells in a human host. Applicants submit that the amendment of claim 14 to include the term "isolated" before "cell" in the first line of the claim obviates this rejection. As such, the rejections under §101 of claims 15 and 16, which depend from amended claim 14, are similarly obviated. Applicants respectfully request that the rejections of claims 14-16 under 35 U.S.C. §101 be withdrawn.

Rejections under 35 U.S.C. §112

A. Rejections under 35 U.S.C. §112, first paragraph:

Claims 1-18 stand rejected under 35 U.S.C. §112, first paragraph, for alleged lack of enablement.

The Examiner states that the specification is enabling for Tet regulation. This is acknowledged. The Examiner questions support as to non-Tet regulatory methods. Non-tet-based regulatory methods are disclosed at page 11, lines 16-27. Specifically, drug-regulatable promoters suitable to the invention in addition to the tet promoter include those regulated by glucocorticoid steroids, sex hormone steroids, LPS and IPTG. Yeast-derived regulatable systems include those based on GAL4 and LEU3 DNA binding proteins and binding elements that are functional in mammalian cells. It is submitted that 35 U.S.C. § 112, first paragraph, does not require that the specification describe in detail for all embodiments of a claimed invention tools that are already available in the prior art.

The Examiner argues that the specification does not enable obtaining a therapeutic effect in a mammal, as required by claims 11 and 12, stating : "At the time of filing, it was unpredictable whether the administration of DNA encoding immunogenic polypeptides against tumors would have a therapeutic result."

The Examiner refers to Ross et al. (1996, Hum. Gene Ther. 7: 1781-1790) as teaching that "the ex vivo approach used to treat tumors resulted in only one melanoma patient who might be considered to have had a clinical response, however it may have occurred spontaneously because melanoma is known to regress spontaneously."

Applicants respectfully submit that this characterization is only *partially* correct: The passage mentioned by the Examiner states "Although some of these studies provided evidence of an immunological response to the intervention, and many directly injected metastatic nodules are said to have regressed, there is only one patient to date who might be considered to have had a significant systemic clinical response..... He has been tumor free for 7 months." The passage does mention spontaneous regression of melanomas, and concludes "Thus, success in a single patient does not imply the general utility of this approach."

The term "therapeutic effect" is defined on page 9, lines 27-29 of the specification as "capable of ameliorating the symptoms or conditions, e.g., of a disease by at least 10%, preferably by 20-50% and most preferably, by 100%". Similarly, "anti-tumor effect" is defined on page 3, lines 23-25 of the specification as "capable of decreasing the size of a tumor by at least 10%, preferably by 20-50% and most preferably by 100%". That is, the definitions of therapeutic effect and anti-tumor effect in the specification call for a 10% minimum amelioration of symptoms or conditions of disease, or tumor size, respectively. The passage cited by the

Examiner is less than optimistic regarding curing cancer, but clearly states reduction in tumor size in a number of cases. Applicants' claims do not refer to curing cancer.

Therefore, Applicants submit that rather than supporting unpredictability of the efficacy of the methods of the present invention, the Ross et al. reference supports the idea that a therapeutic or anti-tumor effect according to the definitions in the specification may be achieved.

The Examiner also refers to Verma et al. (1997, Nature 389: 239-242) for the teaching that "the in vivo approach of gene therapy is unpredictable because of an inability to deliver genes efficiently and to obtain sustained expression". Applicants submit that the reference states: "Thus far, the problem has been an inability to deliver genes efficiently and to obtain sustained expression". This is said, however, in the context of gene therapy to replace a missing function (e.g., factor IX for hemophilia). Applicants submit that this does not apply to a situation where one wishes to reduce a tumor size. Expression need only be sustained long enough to reduce the tumor size, as opposed to the need for life-long replacement of a clotting factor. Therefore, Applicants submit that the Verma et al. reference does not support unpredictability of the presently claimed invention.

The Examiner also relies upon the non-prior art publication by Alvarez-Vallina et al. (1999, Hum. Gene Ther. 10: 559-563) to support his argument of the unpredictability of the claimed methods as of 1999. The Examiner states that the reference teaches "that activation in the Jurkat model of expressing chimeric TCR parallels the activation of normal T cells but that analysis of T cell response over a period of time is required to determine the applicability of cells expressing chimeric TCR". The Examiner concludes that neither the specification nor the teachings of the art "provide adequate guidance indicating cells expressing chimeric TCR have any therapeutic or anti-tumor effect as claimed. Without such guidance, it would require one of skill in the art undue experimentation to determine the parameters required to obtain a therapeutic or anti-tumor effect".

Applicants submit that the Alvarez-Vallina et al. reference does not teach that analysis of T cell response over a period of time is required to determine the applicability of cells expressing chimeric TCR. Rather, the reference states "However, we believe our model does closely parallel the activation of normal T cells because it was shown previously that signals mediated by the ζ chain in immature thymocytes and activated T cells can mimic signaling activities of the intact TCR-CD3-ζ complex. Further detailed analysis of primary T cells at different stages of the immune response will nevertheless be required to confirm and further explore the role of TCR density changes in the "maturation" of T cell responses". There are at least two major reasons that the Alvarez-Vallina et al. reference does not support the Examiner's unpredictability argument with respect to the invention of claims 1-18.

First, Applicants submit that stating that there is more analysis to be performed does not invalidate the major conclusion of the reference, that conclusion being that the responsiveness of T cells to a cell surface antigen can be modulated by modulating the density of (chimeric) receptors for that antigen on the T cell surface. The passage cited by the Examiner is merely the

scientists' admission that they do not know everything there is to know about the importance of TCR density in the T cell response to antigen, and does not undermine the major conclusion or its importance. Applicants submit that the reference supports the use of regulated amounts of chimeric "T-bodies" for enhancing the efficiency of a tumor attack by introduced engineered cells according to the invention.

Second, Applicants note that although the Alvarez-Vallina et al. reference is co-authored by two of the present inventors, and is related in subject matter, it does not describe the invention as claimed in claims 1-18. The claimed invention relates to the use of a drug-regulatable system to maintain the expression of a recombinant immunogenic polypeptide at a low level in a cell, preferably a leukocyte, until the cell has infiltrated a tumor. In this way the host's immune response to the immunogenic polypeptide is avoided until the cell has infiltrated a tumor. In general, the immunogenic polypeptide is an immunotoxin, and chimeric T-bodies may *additionally* be expressed by the cell to enhance the efficiency of tumor attack by the cell expressing an immunogenic polypeptide. See, for example, page 8, line 9 to page 9, line 2. In contrast, the Alvarez-Vallina et al. reference relates only to the modulation of tumor associated antigen-specific chimeric TCR tumor-targeting molecules (T-bodies) on the surface of T cells, which modulation biases T cell activation towards cells expressing higher amounts of a tumor antigen, as a way of avoiding collateral damage to non-tumor tissues that naturally express low levels of a tumor-associated antigen. Considering the differences between the presently claimed invention and the focus of the cited reference, Applicants submit that the conclusions in the reference are not applicable to the predictability of the invention of claims 1-18.

The Examiner also refers to Miller et al., (Hum. Gene Ther. 8: 803-815) as teaching that "the gene regulation system that can be applied to gene therapy in humans is yet unknown". The Examiner states that "Applicants do not demonstrate regulating any genes in humans or in any art recognized in vivo model," and thus do not enable regulating expression of a transgene in a mammal as claimed, particularly not by altering the concentration of a regulatory drug.

Applicants submit that Miller actually states "Hence, efforts are still under way to produce the perfect, human applicable gene regulation system." The instant claims do not require that the drug regulatable system.

Applicants submit that the specification acknowledges the statements made in Miller et al. regarding the drawbacks of the tet regulated system (known as of May 1, 1997), and states that the system described in the specification addresses many of these. Specifically:

"This positive feedback bidirectional promoter system allows for high maximal levels of expression and low levels of tetracycline repressed basal expression when expressed transiently or when stably integrated **into a variety of cell lines**" (p. 14, lines 9-12; emphasis added).

Further, the specification states that the system:

"allows for regulation of both the activity and level of expression of the tetracycline-sensitive DNA-binding expression-regulating polypeptide. As a result of this dual regulation, the amount and activity of the tetracycline-sensitive DNA-binding expression-regulating polypeptide can be kept sufficiently low. Consequently, stable clones expressing this regulatory polypeptide will survive because there are no cytotoxic effects caused by overproduction of this protein. This improved methodology for down regulating the amount and activity of the tetracycline-sensitive DNA-binding expression-regulating polypeptide will also facilitate the production of cells expressing a tet O-linked sequence encoding a toxic protein" (page 14, lines 13-21).

The Examiner also states with regard to claim 10, that Miller teaches "that it is unpredictable what cells the tetracycline system may be applied to," and that in vitro studies in a T cell line "do not correlate the results obtained to other cell lines such that any cell line is enabled." Again, Applicants submit that the cell-specific nature of the Tet system (or lack thereof) is addressed in the specification, at the passages quoted above (see text in bold).

In view of the above remarks regarding the alleged unpredictability of the claimed inventive methods and compositions, and in view of the teachings of the specification, applicants submit that the practice of the invention of claims 1-18 is sufficiently described as to enable one of skill in the art to make and use the claimed invention in the full scope of the claims, with a reasonable expectation of success.

The Examiner has also rejected claim 1 under 35 U.S.C. §112, first paragraph for encompassing, but allegedly not enabling cells naturally expressing a nucleic acid sequence encoding an immunogenic protein operably linked to a drug-regulatable promoter by any means other than by transfection. The Examiner states that it is not clear that any naturally occurring cells with a drug-regulatable promoter exist or how to obtain such cell other than by transfection. Applicants submit that the amendment of claim 1 to specify that the nucleic acid sequence operably linked to a drug-regulatable promoter is recombinant is sufficient to overcome this ground of rejection and respectfully request that the rejection of claim 1 on this basis be withdrawn.

Claims 4-6 are rejected under 35 U.S.C. §112, first paragraph since the claims allegedly do not clearly state that the immune response is a result of the expression of the immunogenic polypeptide. Applicants respectfully submit that the Examiner is misinterpreting the claimed invention. Applicants submit that the substance of the claim is **not** directed to *making* an immune response, *inducing* circulating antibodies, or *making* immunocompetent memory cells, but rather to performing the method of claim 1 in a mammal that, **prior to the introduction of the cell to the mammal**, has made an immune response (amended claim 4), has circulating antibodies to the immunogenic peptide (amended claim 5), or has immunocompetent memory cells specific for the polypeptide (amended claim 6). The Examiner's attention is called to page 22, lines 6-12, which state:

"Accordingly, the method of the invention is typically performed in a mammal **which has already made** an immune response to the immunogenic polypeptide and who may have, for example, circulating antibodies which react with, or immunocompetent memory cells specific for, the immunogenic polypeptide"(emphasis added).

Therefore, Applicants submit that dependent claims 4-6 are not directed to a method of inducing an immune response, but rather, to a method of regulating in a mammal the expression of a nucleic acid sequence encoding a polypeptide which is immunogenic in the mammal, the method comprising introducing into the mammal a cell comprising the nucleic acid sequence encoding the immunogenic polypeptide, said sequence being operably linked to a drug-regulatable promoter, and altering the concentration of regulatory drug to which the cell is exposed in a mammal that, prior to administration of the cell, has made an immune response to (amended claim 4), has circulating antibodies which react with (amended claim 5), or has immunocompetent memory cells which are specific for (amended claim 6) the immunogenic polypeptide. Therefore, Applicants respectfully submit that the rejection of claims 4-6 under §112, first paragraph for the lack of a clear statement that the immune response is a result of the expression of the immunogenic polypeptide is incorrect and request that the rejection be withdrawn.

Claims 7-9 are further rejected under 35 U.S.C. §112, first paragraph. The Examiner states that administration of cells which have been regulated in vitro to express different levels of protein is not a method of regulating the expression of a nucleic acid sequence in a mammal as claimed because the "regulating" does not take place in the mammal. This is incorrect. As the claims state, "the expression of the immunogenic polypeptide is substantially inhibited in vitro", and the "expression of the immunogenic polypeptide reaches a maximum level in the mammal after a delay interval"; thus, the regulating does take place in the mammal via the presence or absence of the regulatory drug. Claims 7-9 refer to operative steps in which the inhibitory drug is removed or added. Applicants therefore request the withdrawal of this rejection of claims 7-9.

Claim 7 is further rejected under 35 U.S.C. §112, first paragraph for alleged failure to enable the term "delay interval". Applicants submit that the term "delay interval" is defined in the specification on page 9, lines 17-19 as follows: "The invention is such that it takes a significant period, or a delay interval, (typically 2 to 10 days, preferably 4 days or longer) for the cell to move from the fully-repressed state to the fully-expressed state." The specification also provides guidance on page 9, lines 14-17 for determining whether the maximum level of expression (i.e., the fully-expressed state) is attained. Therefore, according to the specification, the delay interval is the time it takes for the expression to go from the fully-repressed to the fully expressed state, and guidance is given for one of skill in the art to determine when one has attained full expression. As such, Applicants submit that the meaning of the term "delay interval" is sufficiently described to enable one of skill in the art to practice the method of the invention of claim 7. Applicants therefore respectfully request that this rejection of claim 7 under 35 U.S.C. §112, first paragraph, be withdrawn.

Claim 13 is rejected under 35 U.S.C. §112, first paragraph because the specification allegedly does not enable using a replicable viral genome or viral vector as claimed. The Examiner states that the specification does not provide any guidance on viral vectors which can be used in the instant invention. Applicants submit that the use of a replicable viral genome or viral vector is taught at page 16, lines 8-15. In particular, the literature references Hofmann et al. and Shockett et al. included in the specification provide guidance on viral vectors suitable for use in the invention. Also, the specification states at page 16, lines 11-14 that "In preferred embodiments, the replicable viral genome comprises substantially that of an adenovirus or a paramyxovirus (which genome may be artificially altered by conventional DNA manipulation techniques). Applicants submit further that it is not necessary for a patent disclosure to teach that which is already known in the art. One of skill in the area of recombinant viral vectors would be able to generate and use a viral vector comprising a nucleic acid sequence encoding an immunogenic polypeptide operably linked to a drug-regulatable promoter based on the disclosure in the specification and the knowledge of those skilled in the art. Applicants therefore request that this rejection of claim 13 under 35 U.S.C. §112, first paragraph be withdrawn.

Claims 14-16 are further rejected under 35 U.S.C. §112, first paragraph. The Examiner states that these claims are not enabled because the specification "does not teach transforming the cells within a host which is encompassed by the claim". Claim 14 and thereby dependent claims 15 and 16 all require "a cell transformed with a nucleic acid sequence encoding a polypeptide which is immunogenic to a mammal". According to the definition on page 7, line 32 to page 8, line 6, ""transformation" refers to methods of introducing nucleic acid sequences into eukaryotic cells *in vitro*, including transfection, transduction, electroporation and cell fusion". Therefore, transformation is, by definition, an *in vitro* process. As such, Applicants submit that the claim does not encompass transforming the cells within a host, and therefore request the withdrawal of this rejection.

Claim 18 is rejected under 35 U.S.C. §112, first paragraph because as written it encompasses transformation in vivo. Applicants submit that the addition of the term "isolated" to the claim as amended is sufficient to obviate this rejection, and therefore request withdrawal of this rejection.

B. Rejections under 35 U.S.C. §112, second paragraph:

Claims 1-18 stand rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite.

Specifically, Claim 1 is rejected for use of the phrase "in a mammal of a nucleic acid sequence" because the mammal is not "of a nucleic acid sequence". Applicants submit that this rejection is obviated by the amendment of claim 1. The amendment changes the first line of claim 1 to read "A method of regulating in a mammal the expression of a nucleic acid sequence encoding..."

Claims 4-6 were rejected because the preamble and the substance of the claims allegedly do not provide a nexus between the two. Applicants submit that the substance of the claim is **not** directed to *making* an immune response, *inducing* circulating antibodies, or *making* immunocompetent memory cells, but rather to performing the method of claim 1 in a mammal that, **prior to the introduction of a cell of the invention**, has made an immune response (claim 4 as amended), has circulating antibodies to the immunogenic peptide (claim 5 as amended), or has immunocompetent memory cells (claim 6 as amended). The Examiner is referred to the discussion of claims 4-6 in the above section responding to §112, first paragraph rejections, for further clarification and support for the amendments in the specification (see for example, p. 22, lines 6-12 of the specification). Applicants submit that in view of the above discussion, the claims as originally written are definite, but that the amendments to claims 4-6 presented herein have been added solely to further clarify the meaning of the claims.

Claims 7-9 were rejected under 35 U.S.C. §112, second paragraph, because the phrase "delay interval" is allegedly not defined in the specification and does not have an art recognized meaning. Applicants submit that the term is defined in the specification on page 9, lines 17-19 as follows: "The invention is such that it takes a significant period, or a delay interval, (typically 2 to 10 days, preferably 4 days or longer) for the cell to move from the fully-repressed state to the fully-expressed state." The specification also provides guidance on page 9, lines 14-17 for determining whether the maximum level of expression (i.e., the fully-expressed state) is attained. Therefore, according to the specification, the delay interval is the time it takes for the expression to go from the fully-repressed to the fully expressed state, and guidance is given for one of skill in the art to determine when one has attained full expression. As such, Applicants submit that the meaning of the term "delay interval" is sufficiently clear as to allow one of skill in the art to determine the limits of the claim. Applicants therefore respectfully request that the rejection of claims 7-9 under 35 U.S.C. §112, second paragraph be withdrawn.

Claim 8 was rejected under 35 U.S.C. §112, second paragraph, because the term "substantially free" is allegedly not defined in the specification and may have various meanings in the art. Applicants submit that this rejection is obviated by the amendment of claim 8 to read "[t]he method of claim 7, wherein expression of the immunogenic polypeptide is inhibited in vitro by exposure of the cell to the regulatory drug, and wherein expression in the mammal is induced after a delay interval following removal of the regulatory drug exposure". Support for this amendment may be found on page 18, lines 27-30, which read "[g]enerally it is preferred that the presence of the regulatory drug inhibits expression of the immunogenic polypeptide, as subsequent *removal of the cell from regulatory drug exposure* normally gives a longer lag phase or delay before induction of expression of the immunogenic polypeptide" (emphasis added). Further support is also found on page 19, lines 16 and 17, which reads "... time course for resumption of activity of the tetracycline regulated promoter *following removal of the particular analogue*" (emphasis added).

Claim 9 is rejected under 35 U.S.C. §112, second paragraph, as indefinite for use of the phrase "mammal of the regulatory drug" because the mammal is not "of the regulatory drug". Applicants submit that the amendment of claim 9 to read in part "wherein expression in the

mammal is induced after a delay interval by administration *of the regulatory drug to the mammal*" (emphasis added) obviates this rejection.

Claim 9 was also rejected under the same statute as indefinite for use of the phrase "substantial absence", which allegedly lacks a definition in the specification. The Examiner's attention is called to page 5, lines 7-11, which state:

> "Substantial absence means an amount that is undetectable by immunological or enzymatic methods of detection. In particular, the substantial absence of a regulatory drug refers to an amount or [sic] regulatory drug that does not stimulate an increase or decrease in the expression of an immunogenic polypeptide sequence that is operably linked to a promoter that is regulated by this same regulatory drug."

Thus, a "substantial absence" is defined with respect to the expression of an immunogenic polypeptide; if an increase or decrease in the immunogenic polypeptide cannot be detected, then this indicates a substantial absence of the drug that regulates the drug-regulated promoter. Applicants submit that this passage clearly defines what is meant by "substantial absence" of a drug, and therefore respectfully request withdrawal of this ground of rejection of amended claim 9.

Claim 16 was rejected as indefinite for use of the term "plurality of a cell of claim 14", since a plurality cannot be a cell as claimed. Applicants submit that this rejection is obviated by the amendment of claim 16 to read "[a] composition comprising a plurality of cells of claim 14 and a physiologically acceptable diluent."

Rejections under 35 U.S.C. §102(b):

Claims 14-17 stand rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Taichman et al. The Office Action states that Taichman et al. teaches "producing a plasmid encoding a tyrosine kinase operatively linked to an inducible MT-I promoter and transfecting T cells with the plasmid". The Office Action also states that the transgene "is controlled by altering the concentration of zinc in the media", and that "since all proteins are immunogenic, the tyrosine kinase is an immunogenic polypeptide as claimed". Finally, the Office Action states that "the media taught by Taichman et al. "is considered equivalent to the physiologically acceptable diluent claimed". Applicants submit that Taichman et al. does not anticipate the invention of amended claims 14 and 16 or claims 15 and 17.

Amended claim 14, and claims 15-16 that depend from it, relates to a cell transformed with a nucleic acid sequence encoding a polypeptide which is immunogenic to a mammal, the nucleic acid sequence being operatively linked to a drug-regulatable promoter, such that expression of the immunogenic polypeptide by the cell may be controlled by altering the concentration of regulatory drug to which the cell is exposed *in a mammal*. Applicants submit that Taichman et al. does not teach controlling the expression of an immunogenic polypeptide by altering the concentration of regulatory drug to which the cell is exposed *in a mammal*, rather

teaching only modulation of thymidine kinase activity in tissue culture. Therefore, Applicants submit that Taichman et al. does not anticipate the invention of claims 14-16.

Claim 17, which relates to a method of making a physiological composition, requires "mixing the selected cells with a physiologically acceptable diluent". Applicants submit that, contrary to the conclusion stated in the Office Action, cell culture medium is not, nor is it equivalent to, a physiologically acceptable diluent according to the definition in the specification at page 6, lines 10-12. The definition states: "Physiologically acceptable diluent means water, phosphate buffered saline, or saline, and further may include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are materials well known in the art." This definition clearly does not include cell culture medium, and, as such, Applicants submit that Taichman et al. does not anticipate claim 17.

In view of the above, Applicants respectfully request that the rejections of claims 14-17 under 35 U.S.C. §102(b) be withdrawn.

In view of the preceding amendments and remarks, Applicants submit that all rejections and objections have been addressed, and as such, submit that claims 1-18 are in condition for allowance and respectfully request such action by the Examiner.

Respectfully submitted,

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Date

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